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(54) **Vitamin E extraction**

(57) Tocopherols and tocotrienols are
extracted from the leaves of the palm
tree, banana, pineapple and/or sugar
cane with one or more organic
solvents. Further treatment of the

obtained extract solution by means of
low-temperature fractionation,
adsorption by use of an adsorbent or
molecular distillation, or a
combination of these treatments,
gives a concentrate of tocopherols
and tocotrienols.

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SPECIFICATION

Method for separating tocopherols and tocotrienol

This invention relates to a method for separating tocopherols and tocotrienol, and more particularly it relates to a method for extracting tocopherols and tocotrienol contained in leaves of palm tree, banana or the like with an organic solvent or solvents and concentrating said substances. 5

It is known that tocopherols and tocotrienols have a vitamin E activity, and they have long been utilized as an antisterility drug for animals. It has been disclosed lately that these substances further possess an antioxidizing action for oil- or fat-derived foods as well as a medicinal action of preventing adult diseases and senility, and their demand for use as food additives, pharmaceuticals, nourishing 10 foods and cosmetics has increased sharply.

Hitherto, tocopherols and tocotrienols have been separated from deodorized distillates of soybean oil, cotton seed oil or the like. However, because of their relatively low content in said raw materials and also the complicated processes for separation and refining, said substances have been relatively costly and also their supply could hardly meet the rushing demand.

If it is made possible to separate tocopherols and tocotrienols from more inexpensive natural products by a more simple process, then it will become possible to supply said substances at lower cost and in greater amounts to well meet the growing demand.

The present invention has been contemplated under these circumstances and its object is to provide a method for extracting tocopherols and tocotrienol from inexpensive natural products by a 20 simple procedure.

Another object of this invention is to provide a method for concentrating extracts of tocopherols and tocotrienol obtained from such inexpensive natural products.

Thus, the present invention, in one aspect thereof, is characterized by extracting tocopherols and tocotrienol from leaves of palm tree, banana, pineapple or sugar cane (*Saccharum officinarum*) by 25 treating leaves of one of said plants or mixed leaves of two or more of said plants with a single organic solvent or a mixture of organic solvents.

In another aspect, the present invention is characterized by treating leaves of one of palm tree, banana, pineapple and sugar cane or mixed of leaves of two or more of said plants with a single or mixed organic solvent and further treating the extract by means of low-temperature fractionation, 30 adsorption by use of an adsorbent or molecular distillation, or a combination of said treating means, to thereby concentrate tocopherols and tocotrienol.

The tocopherols to be extracted in this invention are α -tocopherol, β -tocopherol, γ -tocopherol and δ -tocopherol. The tocotrienol, which is another substance to be extracted in this invention, is α -tocotrienol. Of these substances, β - and δ -tocopherols and α -tocotrienol are minor components and α - 35 tocopherol which has the strongest vitamin E activity is the principal component.

Shown in the following table are contents of said substances in the leaves of palm tree, banana or sugar cane.

	Tocopherols				Toco- trienol	Total
	α	β	γ	δ	α	
Palm tree	14,805	44	84	12	18	14,963
Banana	1,687	13	1,250	12	—	2,952
Sugar cane	3,984	—	402	—	—	4,386

Unit: $\mu\text{g/g}$ of extract

The leaves of palm tree, banana, pineapple or sugar cane to be used for extracting said objective substances in this invention include those of said plants which are growing or after harvesting or reaping. For instance, leaves cut out when harvesting fruits of said plants or leaves of sugar cane cut down when reaping it may be used. Also, leaves of all species of palm tree, banana, pineapple and sugar cane can be used. These leaves are usually chopped, crushed or pulverized, and in some cases 45 further dried thereafter. In order to facilitate the extraction treatment by an organic solvent or solvents. Also, leaves of only one of said plants, for example, palm tree alone, or leaves of two or more of said plants, for example mixed leaves of palm tree and banana may be used for the extraction treatment of this invention. 50

As the examples of the organic solvents usable in this invention, there may be cited aliphatic 55 alcohols such as methanol, ethanol, or isopropanol, aliphatic ethers such as ethyl ether or isopropyl ether, aliphatic ketones such as methyl ethyl ketone, aliphatic carboxylic acid esters such as methyl formate, ethyl formate, or ethyl acetate, aliphatic and aromatic hydrocarbons such as petroleum ether, hexane, heptane, benzene, toluene, or oxylene, and halogenated aliphatic hydrocarbons such as chloroform, methylene chloride, or ethylene chloride. These organic solvents may be used either alone

or in admixture. Also, mixtures of these organic solvents with water-insoluble organic solvents may be used.

The extraction is usually carried out at room temperature, or between 20 and 25°C. For instance, chopped leaves of palm tree, banana, pineapple or sugar cane are immersed in an organic solvent or a mixture of the organic solvents mentioned above and, after 1- to 3-hour standing, the leaves are filtered off to obtain an extract solution of tocopherols and tocotrienol.

Further, according to this invention, the thus obtained extract solution of tocopherols and tocotrienol can be concentrated by subjecting said extract solution to low-temperature fractionation, adsorption by use of an adsorbent or molecular distillation or a combination of such treatments.

The low-temperature fractionation is accomplished by cooling the extract solution of tocopherols and tocotrienol to a temperature of -10 to -40°C, this cooling being usually conducted after further adding an organic solvent or solvents for facilitating the fractionation, and separating an organic solvent insoluble portion from a soluble portion, followed by the removal of the organic solvent(s), as by distillation, from the soluble portion to obtain concentrated tocopherols and tocotrienol as a residue.

The adsorption treatment by use of an adsorbent in this invention can be accomplished by first adding a non-polar organic solvent to said extract solution of tocopherols and tocotrienol, passing the solution through an alumina- or silica-packed column, subsequently passing a non-polar organic solvent and/or an alcohol, an ether, a ketone, an ester, an aromatic compound and/or an organic chloride either alone or as a mixed solution, and then removing the solvent(s) from eluted fractions to obtain a tocopherol and tocotrienol concentrate as a residue. Hydrocarbons such as petroleum ether, hexane, or heptane may be used as the non-polar organic solvent in this treatment. Alternatively, the treatment may be accomplished by passing the extract solution and a mixed solution of non-polar and polar organic solvents through a column packed with an ion exchange resin instead of alumina or silica, then further passing an acidic organic solvent such as an acetic acid-ethanol solution, washing the obtained fraction with water and distilling off the organic solvents, whereby a desired tocopherol and tocotrienol concentrate is given as a residue.

Further, in accordance with this invention, a similar concentrate of tocopherols and tocotrienol can be obtained by subjecting said extract solution to molecular distillation under a vacuum of 10^{-2} to 10^{-3} mmHg or below and recovering a fraction which distills out at 190° to 230°C.

As described above, it is possible according to this invention to separate tocopherols and tocotrienol by a simple operation involving extraction with an organic solvent or solvents by using inexpensive material such as leaves of palm tree, banana, pineapple or sugar cane.

Further, in accordance with this invention, a concentrate of tocopherols and tocotrienol can be obtained with ease by subjecting the extract solution of tocopherols and tocotrienol to low-temperature fractionation, adsorption treatment or molecular distillation.

It will thus be appreciated that the present invention makes it possible to produce tocopherols and tocotrienol, which have a vitamin E activity, at low cost and in great quantities and can well comply with the expanding demand for said substances.

The present invention will be described in further detail hereinafter in accordance with the examples thereof.

Example 1

2 kg of palm leaves (water content: 42%) were chopped and extracted with a hexane-ethanol (1:1 v/v) mixed solution at room temperature (22—24°C) to obtain 102 g of an extract. 800 ml of ethanol was added to this extract and the mixture was mixed well by shaking and then cooled to -20°C to fractionate a soluble portion. Ethanol was distilled off from said soluble portion to obtain 15 g of a residue. High performance liquid chromatography of this residue (1) showed that it contained 81,000 µg/g of α -tocopherol, 350 µg/g of β -tocopherol, 4,240 µg/g of γ -tocopherol and 200 µg/g of γ -tocotrienol, or total 85,790 µg/g of tocopherols and tocotrienol combined.

Examples 2

1 kg of sugar cane leaves (water content: 73%) were chopped and extracted by using a hexane-ethanol (1:1 v/v) mixed solution at room temperature (20—22°C) to obtain 15 g of an extract. This extract was well mixed with 200 ml of ethanol by stirring and then cooled to -20°C to fractionate a soluble portion, followed by distilling-off of ethanol from said soluble portion to obtain 3 g of a residue. High performance liquid chromatography conducted on said residue showed an α -tocopherol content of 12,000 µg/g and a γ -tocopherol content of 4,500 µg/g, or a total tocopherol content of 16,500 µg/g.

Example 3

10 g of residue (1) obtained in Example 1 was dissolved in 50 ml of an ethanol-hexane (1:1) mixed solution and the mixture was passed through a layer of Amberlite IRA 401, an OH type basic anion exchange resin, packed in a 5 cm-diameter and 100 cm-long glass-made chromatographic tube to a height of 50 cm to have tocopherols and tocotrienol adsorbed in said layer, and then 300 ml of hexane was flown therethrough to let the neutral matter run away. Then 200 ml of a 5% acetic acid-

ethanol solution was passed and the solvents were distilled off from the eluate to obtain 0.8 g of a tocopherol concentrate (purity: 65%).

Example 4

1 kg of pineapple leaves (water content: 45%) were chopped and extracted with acetone at room temperature (23—24°C) to obtain 45.2 g of an extract. This extract was dissolved in 400 ml of ethanol and then cooled to -20°C to fractionate a soluble portion. Then ethanol was distilled off from said soluble portion to obtain 8.6 g of a residue. This residue contained 48,000 µg/g of α -tocopherol, 12,800 µg/g of γ -tocopherol, 650 µg/g of α -tocotrienol and 1,100 µg/g of γ -tocotrienol, or total 62,550 µg/g of tocopherols and tocotrienols.

Example 5

20 kg of banana leaves (water content: 65%) were chopped and extracted by using a hexane-ethanol (1:1 v/v) mixed solution at room temperature (24—25°C). The extract solution contained 22,000 µg of a α -tocopherol and 1,060 µg of γ -tocopherol, totalling 23,060 µg of tocopherols.

Example 6

4 kg of palm leaves (water content: 48%) were crushed down by using a roller and then chopped, and the chopped leaves were added to 12 litres of a hexane-ethanol (1:1 v/v) mixed solution and allowed to stand overnight at room temperature (22—24°C), followed by the fractionation of the extract solution to obtain 186 g of an extract. This extract was well mixed with 1 litre of methanol by shaking and cooled to 0°C to fractionate a soluble portion. Methanol was distilled off from this soluble portion to obtain 48 g of a residue. To 20 g of this residue was added 20 g of refined palm oil and the mixture was subjected to molecular distillation under 5×10^{-3} mmHg to fractionate the mixture into four fractions: a first fraction at 190°C or below, a second fraction at 190—200°C, a third fraction at 200—220°C and a final residue. The tocopherol content of each fraction was as follows: 23.5% in the first fraction, 8.1% in the second fraction, 2.5% in the third fraction and 0.6% in the final residue.

Example 7

2.3 kg of palm leaves (water content: 42%) were chopped and extracted with a hexane-ethanol (1:1 v/v) mixed solution at room temperature (22—24°C) to obtain 116 g of an extract. 51 of hexane-ethanol (1:1 v/v) mixed solution of hexane was added to this extract and mixed well. This mixture was washed with large amount of hot water to remove water soluble matters from said mixture and then dried completely followed by cooling to -20°C to fractionate a hexane soluble portion. This hexane soluble portion was passed through a silica-packed column (Bio Sil A, 100—200 mesh), subsequently hexane was passed. Hydrocarbons were eluted first and then α -tocopherol was eluted by hexane. By collecting α -tocopherol fraction and removing solvent from said fraction, 2.5 g of α -tocopherol concentrate was obtained as a residue. A concentration and a recovery of α -tocopherol of said residue were 68.4% and 83.3%, respectively.

Claims

1. A method for separating tocopherols and tocotrienol which comprises treating leaves of palm tree, of banana, of pineapple and/or of sugar cane either alone or in admixture with a single or mixed organic solvent and extracting tocopherols and tocotrienol.
2. The method for separating tocopherols and tocotrienol according to Claim 1, wherein the organic solvent is selected from the group consisting of aliphatic alcohols, aliphatic ethers, aliphatic ketones, aliphatic carboxylic acid esters, aliphatic hydrocarbons, aromatic hydrocarbons and halogenated aliphatic hydrocarbons.
3. The method for separating tocopherols and tocotrienol according to Claim 1, wherein the leaves of palm tree, of banana, of pineapple and/or of sugar cane are used after chopping or pulverizing them.
4. A method for separating tocopherols and tocotrienol which comprises treating leaves of palm tree, of banana, of pineapple and/or of sugar cane either alone or in admixture with a single or mixed organic solvent, and subjecting the obtained extract solution to one of the following treatments or a combination thereof: low-temperature fractionation, adsorption by use of an adsorbent, and molecular distillation, to thereby obtain a concentrate of tocopherols and tocotrienol.
5. The method for separating tocopherols and tocotrienol according to Claim 4, wherein the organic solvent is selected from the group consisting of aliphatic alcohols, aliphatic ethers, aliphatic ketones, aliphatic carboxylic acid esters, aliphatic hydrocarbons, aromatic hydrocarbons and halogenated aliphatic hydrocarbons.
6. The method for separating tocopherols and tocotrienol according to Claim 4, wherein the leaves of palm tree, of banana, of pineapple and/or of sugar cane are used after chopping or pulverizing them.
7. The method for separating tocopherols and tocotrienol according to Claim 4 wherein the adsorbent is silica, alumina or an ion exchange resin.

8. The method for separating tocopherols and tocotrienol according to Claim 4 wherein the adsorption treatment by use of an adsorbent is conducted after adding a non-polar organic solvent to the extract solution.

9. A method for separating tocopherols and tocotrienol as claimed in claim 1 and substantially as described in any of Examples 1 to 7.

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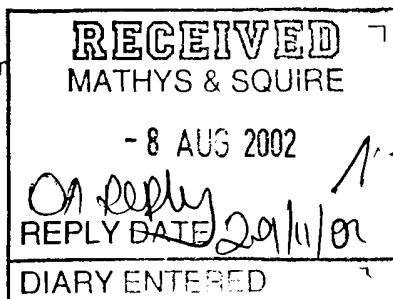
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Application No. 00 907 000.4-2117	Ref. GWS/23761	Date 29.07.2002
Applicant Archer-Daniels-Midland Company		

Communication pursuant to Article 96(2) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(1) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 78(2) and 83(2) and (4) EPC.

One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (Rule 36(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Article 96(3) EPC).



FRITZ M
Primary Examiner
for the Examining Division

Document **AR3**
Appl. No. 09/360,947

Enclosure(s): 4 page/s reasons (Form 2906)



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Date

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Anmelde-Nr.:
Application No.:
Demande n°:

00 907 000.4

The examination is being carried out on the **following application documents**:

Text for the Contracting States:

AT BE CH LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Description, pages:

1-79 as originally filed

Claims, No.:

1-47 as originally filed

Drawings, sheets:

1/1 as originally filed

1. The following documents (D1-D4) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: US-A-5 487 817 (FIZET CHRISTIAN) 30 January 1996 (1996-01-30)

D2: US-A-5 512 691 (BARNICKI SCOTT D ET AL) 30 April 1996 (1996-04-30)

D3: GB-A-2 117 381 (KOGYO GIJUTSUIN) 12 October 1983 (1983-10-12)

D4: EP-A-0 171 009 (HENKEL CORP) 12 February 1986 (1986-02-12)

2. The present case describes methods for separating a tocopherol from a mixture comprising at least one tocopherol, a fatty acid and an esterifying compound (claims 1-39 and 43-47) and compositions produced by the processes of claim 1, claim 25 and claim 38 (claims 40-42).
3. The feature rendering novelty to the process according to the present case is the extraction step which is carried out by using a polar organic solvent which is not a neat alcohol.

The first three process steps are, as was also acknowledged by the applicant, well



known in the art (cf. e.g. D1 and D2). The result of these three reaction steps is a composition with an enriched content of tocopherols.

In the process disclosed in the present case this composition is extracted with a polar organic solvent which is not a neat alcohol which then leads to a composition being more concentrated with respect to tocopherols.

It is not clear in how far the compositions of claims 40-42 which are only defined by a process for their production can be distinguished from other compositions comprising tocopherols which have been described in the art. The applicant is invited to comment on this point.

It is - in this respect - noted that the novel process employed for their production is not a technical feature which would render the subject-matter of claims 40-42 novel (cf. T 150/82).

The novelty of claims 1-39 and 43-47 is acknowledged (Article 54 EPC).

4. In view of D1 and D2 which are the closest prior art the problem of the present case must be formulated as to provide a method which is suitable to further increase the concentration of tocopherols in a composition comprising them.

As was extensively discussed by the applicant in the description, the extraction with methanol (DE 3615029) or caustic methanol (US Patent No. 4550183) or supercritical CO₂ (JP60048981 and JP60149582) was disclosed, however all of methods have serious drawbacks.

In order to solve the problem the skilled man simply had to choose other solvents which are also suitable as extraction agents which do not have the drawbacks as the methods of above.

As various documents (D3-D4) teach that tocopherols can be extracted by polar organic solvents which are not alcohols, the solution proposed must be considered trivial and well within the ability of the ordinary skilled man.



An inventive step in the sense of Article 56 EPC can therefore not be acknowledged for the subject-matter of claims 1-39 and 43-47.

If it could be demonstrated that the subject-matter of claim 40-42 is indeed novel in the sense of Article 54 Epc, the following is stated with regard to the presence of an inventive step:

The inventive step of a chemical product can only reside in a process for its preparation, when a technical prejudice to its production or insurmountable difficulties in its production were believed to exist (cf. Guidelines C-IV, 9.8(d)).

As this is not the case here, an inventive step is not acknowledged for the subject-matter of claims 40-42 (Art. 56 EPC).


5. Furthermore the following amendments should be carried out:

The term "substantially" used in claims 1, 43, 45 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 84 EPC).

The unit "torr" expressed in claims 5,6,13-16 and on p. 26, lines 27-28, p. 29, lines 13-14,25-26, p. 30, lines 19-21 as well as the units "mm Hg" employed in ex. 1 do not meet the requirements of Rule 35(12) EPC and should be replaced by the appropriate SI units (cf. the Guidelines, C-II, Annex 1). The present expressions should, however, be retained in parentheses after the replacement expressions.

The term "does not comprise" employed in claim 39 (p. 89, line 3) should be substituted by "is not" (cf. claim 1 and also p. 37, lines 6-9).

The vague and imprecise expression "spirit" in the description on page 79, lines 8 and 11 implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity of the claims (Article 84 EPC) when used to interpret them (see the Guidelines, C-III, 4.3a).



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Anmelde-Nr.:
Application No.: 00 907 000.4
Demande n°:

The claims refer to a method for separating tocopherols from a tocopherol-containing mixture. In contrast thereto the description also refers to tocopherols (i.e. tocopherols + tocotrienols) and tocotrienols which is unsearched subject-matter.

The applicant is - with reference to Rule 86(4) EPC - requested to delete the unsearched subject-matter from the description.

The subject-matter to be excised may be made the subject of one or more divisional applications. The divisional applications must be filed directly at the European Patent Office in Munich or its branch at The Hague and in the language of the proceedings relating to the present application, cf. Article 76(1) and Rule 4 EPC. The time limit for filing divisional applications (Rule 25(1) EPC) must be observed.

6. To meet the requirements of Rule 27(1)(b) EPC, the documents D3-D4 should be identified in the description and the relevant background art disclosed therein should be briefly discussed.
7. When filing amended claims the applicant should at the same time bring the description into conformity with the amended claims. Care should be taken during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Article 123(2) EPC).

In order to facilitate the examination of the conformity of the amended application with the requirements of Article 123(2) EPC, the applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.

If the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed.